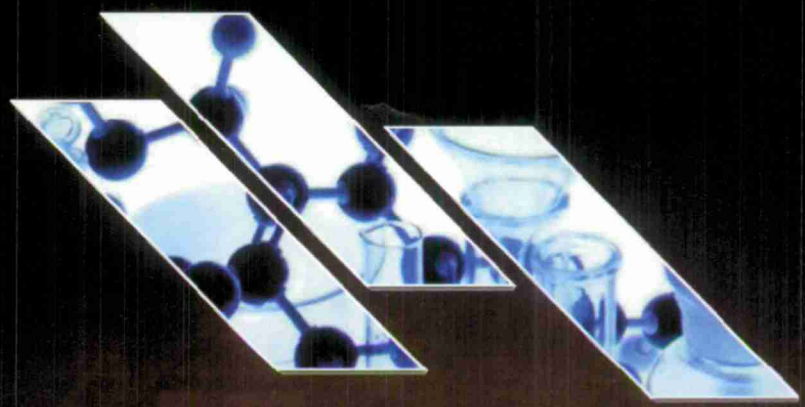
EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-926

PHASE 1 TESTING OF BIOFLASH TECHNOLOGY FOR WHITE POWDER IDENTIFICATION



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June 2012

U.S. ARMY RDECOM

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-06-2012	Final	Aug 2009 - Aug 2010

4. TITLE AND SUBTITLE

Phase I Testing of BioFlash Technology for White Powder Identification

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Wallace, Lalena; Rastogi, Vipin, K; Gardner, Warren; and Sickler, Todd

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRB-D, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-926

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Department of Homeland Security, Science and Technology Directorate, 245 Murray Lane, SW, Building 410, Washington, DC 20528

10. SPONSOR/MONITOR'S ACRONYM(S)
DHS S&T

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Phase I proof-of-concept testing of a mock-up of the cellular analysis and notification of antigen risks and yields (CANARY) bioagent-identification system was conducted at the U.S. Army Edgewood Chemical Biological Center. This testing was sponsored by the U.S. Department of Homeland Security Science and Technology Directorate in support of the TechSolutions Program, which seeks to rapidly address technology gaps identified by First Responders. The mock-up was based on the CANARY technology developed by the Massachusetts Institute of Technology Lincoln Laboratory. The two main objectives of this testing were (1) to determine the limit of detection for the test bed system for powdered spores of *Bacillus anthracis* and *Bacillus subtilis*, and (2) to determine if common nonhazardous white powders trigger a false positive response or subsequently interfere with the ability of the system to detect real agents. The findings are summarized as follows: (1) the powder collection apparatus was used to successfully collect the powdered samples into the testing dises; (2) low levels of known agent were consistently detected with the system; and (3) some of the nonhazardous white powders yielded unexpected results because the white powders interfered with the detection system.

15. SUBJECT TERMS

White powder identification	*B. subtilis*	*B. anthracis*
Spore detection	CANARY	First Responder
Bioagent identification	Biodetection	Bioagent detection

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					Renu B. Rastogi
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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EXECUTIVE SUMMARY

The U.S. Army Edgewood Chemical Biological Center (ECBC) conducted the Phase I proof-of-concept testing of a mock-up of the cellular analysis and notification of antigen risks and yields (CANARY) bioagent-identification system developed by the Massachusetts Institute of Technology Lincoln Laboratory ([MIT LL] Lexington, MA). The U.S. Department of Homeland Security Science and Technology Directorate (Washington, DC) sponsored the testing in support of the TechSolutions Program, which seeks to rapidly address technology gaps identified by First Responders.

The system, as tested by ECBC, was composed of three components: a powder collection apparatus (PCA) engineered by MIT LL, a commercially available BioFlash instrument (Innovative Biosensors, Inc., Rockville, MD), and a laptop, supplied by ECBC, interfaced directly with the BioFlash instrument, which was used for retrieving raw data files. Because a prototype of the concept handheld system was not available during Phase I, testing was conducted using the proof-of-concept test bed. The two main objectives of this testing were to determine: (1) the limit of detection (LOD) for the test bed system for powdered spores of *Bacillus anthracis* and *Bacillus subtilis*; and (2) if common nonhazardous white powders trigger a false positive response or subsequently interfere with the ability of the system to detect real agents.

The LOD testing was conducted with two preparations each of *B. subtilis* and *B. anthracis*, a professional preparation (PP) and a lesser preparation (LP). The PP was washed, milled, and fluidized. It represented the quality of material that could be prepared by skilled personnel. Thc LP was prepared crudely to represent material that could be prepared by personnel with little or no expertise. For each of the four spore preparations, four decreasing sample weights were tested to determine if the BioFlash instrument could positively detect the presence of agent. The four sample weights were 10, 1, 0.1 (trace amount [TA]) mg, and a residual amount (RA) of unspecified weight. With the exception of the RA samples, all the sample weights were tested in triplicate.

The results of the LOD testing indicated that the system was able to detect known agent samples at all sample weight levels. Each weight sample series for the spore preparations included one or two samples with residual amounts of powdered agent, referred to as RA samples. For an RA sample, a foam swab, similar to the kind used by a First Responder, was used to swab the interior of the TA sample vial after the TA sample had been tested. The purpose of the RA sample was to see if any residual powder, which was not discernible by visual inspection, could be picked up by the swab, introduced into the disc, and subsequently detected by the BioFlash instrument. Agent was detected by the system for three of the four RA samples tested. Of all the LOD samples tested, only one known agent sample returned a "No Agent" result. Five known agent samples resulted in an "Inconclusive" indication. Further analysis of the data revealed that with the exception of one sample agent test, the correct channels were triggered for the specific agent tested. For the No Agent result, an additional channel was erroneously triggered.

For the nonhazardous white powder testing, the following six common white powders were assayed:

- flour
- kaolin
- alconox
- confectionary sugar
- powdered nondairy creamer
- yeast

Approximately 1 mg of each powder sample was tested in triplicate. A positive *B. subtilis* sample was tested between each powder sample to ensure proper functioning of the instrument after the introduction of each powder. Analysis of the nonhazardous white powder testing data revealed that 17 out of 36 or 47% of the results were unexpected. Flour, kaolin, and alconox proved problematic for the detection system because each sample of these powders resulted in unexpected indications. Confectionary sugar, powdered nondairy creamer, and yeast did not appear to interfere with the functioning of the system, and they were correctly identified as nonagent samples.

Most of the unexpected results for the LOD testing and nonhazardous white powder testing may have been due to a high background signal from nonspecific channels. It is believed that several erroneous determinations resulted from mechanical malfunctions of the BioFlash instrument. Additionally, because the discs were prepared with no quality assurance procedures in place, human error could account for some of the unexpected results.

The findings are summarized as follows:

- The PCA was used to successfully collect the powdered samples into the testing discs.
- Low levels of known agent were consistently detectable with the system.
- Some of the results for the nonhazardous white powders were unexpected because the powders interfered with the functioning of the detection system.

Furthermore, a detailed analysis of the raw data revealed that by resetting the parameters to make the algorithm more stringent, the number of unexpected results could be reduced significantly.

PREFACE

The work described in this report was sponsored by the U.S. Department of Homeland Security Science and Technology Directorate ([DHS S&T] Washington, DC). The work was started in August 2009 and completed in August 2010.

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This report has been approved for public release.

Acknowledgment

The authors would like to acknowledge William Deso (DHS) for providing funding for this project.

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PHASE I TESTING OF BIOFLASH TECHNOLOGY FOR WHITE POWDER IDENTIFICATION

1. INTRODUCTION

The testing described in this report was funded by the U.S. Department of Homeland Security, Science and Technology Directorate TechSolutions program (Washington, DC). This program seeks to rapidly address technology gaps identified by First Responders by fielding prototype solutions within 12–18 months. This report details the Phase 1 proof-of-concept testing performed at the U.S. Army Edgewood Chemical Biological Center (ECBC) for the identification of suspect white powder samples, using a mock-up of a handheld cellular analysis and notification of antigen risks and yields (CANARY) detector, developed by the Massachusetts Institute of Technology Lincoln Laboratory ([MIT LL] Lexington, MA) .

The Phase I testing had the following objectives:

- To determine the limit of detection (LOD) for specific powdered agents.
- To determine the effects of introducing samples containing only nonhazardous "hoax" white powder substances to the detection system.

The system tested at ECBC was composed of three components:

- A powder collection apparatus (PCA) engineered by MIT LL
- A commercially available BioFlash instrument manufactured by Innovative Biosensors, Inc. ([IBI] Rockville, MD)
- A laptop supplied by ECBC to collect the raw data values

The device for aerosol collection in the BioFlash instrument was bypassed for the ECBC Phase I testing. Instead, the PCA was used to collect powdered samples into the assay discs.

2. BACKGROUND

Modification of the current BioFlash detector has the potential to advance a First Responder's ability to screen suspect powders and identify hazardous biological materials of concern. The BioFlash instrument uses the proprietary CANARY technology, which is a patented, self-contained system consisting of genetically engineered B cells expressing membrane-bound, pathogen-specific antibodies. Once bound by a specific pathogen, these antibodies lead to the elevation of intracellular calcium (Rider et al., 2003). The calcium triggers the expression of an engineered cytosolic bioluminescent protein, which results in light emission. This light emission is then read by a photomultiplier tube detector in the BioFlash instrument. The light output data are collected by the system software and then loaded into the computer

interface. An algorithm is used to make a determination as to whether or not a biological agent of interest is present, based on the pattern and intensity of the light output values for each channel. Each B cell line is engineered to detect a specific pathogen, so each channel is capable of eliciting a different response depending on which cell line has been loaded in the channel. The discs are set up in a specific manner so that the data can be interpreted by the algorithm. For the Phase 1 testing, all the discs were loaded manually according to the predetermined setup scheme.

An algorithm is used to translate the light-emission signals into a detection indication (e.g., "Agent Found", "No Agent Found"). The algorithm is used to interpret the data and make determinations based on the shape of the light output curve. The first two stages of algorithm processing smooth the curve and produce a fast Fourier transform (FFT) of the data. Positive samples (biosensor + target) have been known to ascribe specific curve shapes and FFTs with certain properties. Different parameters can be changed within the algorithm to alter the interpretation of the data, which can change the final indication made by the system. The parameters, which can be set in the algorithm to get reproducible results for the positive samples, are often target-specific. If the light output curve meets the required values for all the set parameters, the sample producing the curve is determined to be positive.

Examples of changeable parameters include

- True peak window: the period of time in which the light output curve must reach its maximum value
- Minimum run: the number of positive slopes in a row required for a curve to reach its peak light output
- Minimum maximum value: the minimum acceptable value for maximum light output

These parameter examples apply to the smoothed data curve while others are specific to the FFT power spectrum. This power spectrum is a representation of the relative strengths of signal output at different frequencies. Interpretation of the FFT deals with the abstract properties of the curve, including the signal-to-noise ratio and frequency of the primary amount of noise. The parameters of the algorithm are tunable and determined empirically based on experimentally collected data. Optimization for a given target application is often beneficial. Time parameters are modified to increase detection and must be checked to determine their affect on signal specificity.

Because the algorithm tested at ECBC was designed for maximum sensitivity, its modification may be necessary to improve specificity. This is particularly applicable to the ECBC testing because three different cell lines were being tested on each sample disc. Adjusting the algorithm to achieve maximum sensitivity for one cell line could affect the specificity for another. Additionally, the concentration of agent in a barely visible amount of powder was much higher than the reported LODs for this technology; therefore, the signal may have been much higher than expected, and the algorithm may have needed appropriate adjustment.

3. MATERIALS AND METHODS

All testing was conducted at ECBC in a biosafety level 2 laboratory. All powder introductions were conducted in a Class II biological safety cabinet by personnel wearing required protective equipment.

The instrumentation used for testing consisted of the PCA (shown in Figure 1) and BioFlash detector. A total of 91 samples were analyzed during the ECBC testing: 36 samples were analyzed during the nonhazardous powder testing and 55 samples were analyzed to determine the LOD for specific agents.

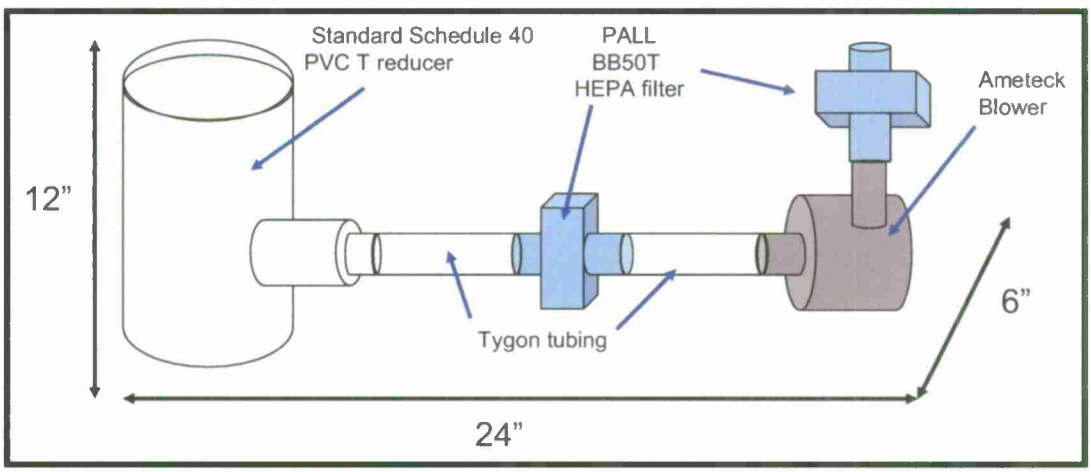

Figure 1. PCA.

3.1 Spore Preparation and Quality Assurance/Quality Control (QA/QC)

ECBC Phase 1 testing was performed with spores of *Bacillus subtilis* and *Bacillus anthracis* Sterne prepared at Dugway Proving Grounds (UT). Two preparations of each spore type were tested: a clean milled and fluidized preparation (professional preparation [PP]) and a crude preparation (lesser preparation [LP]). The PP was washed, milled, and fluidized, and it represented the quality of material that could be prepared by skilled personnel. The LP was prepared crudely to represent material that could be prepared by personnel with little or no expertise.

The spore preparations were enumerated at ECBC upon receipt. A preweighed amount of spores was reconstituted in 1 mL phosphate buffered saline ([PBS] Life technologies, Grand Island, NY). A series of dilutions was made in sterile PBS, and a 100 µL aliquot of each dilution was plated in triplicate on tryptic soy agar ([TSA] BD Diagnostics Systems, Sparks, MD) plates.* The plates were incubated overnight at 37 °C to allow colony growth. After 18–24 h of incubation, the colonies were counted using an automated QCount (Spiral Biotech, Norwood, MA) colony counter unit. The enumeration results are shown in Table 1.

* The TSA plates were prepared by the Biodefense (ECBC) branch members using the powder.

Table 1. Spore Preparation Concentrations

Spore Preparation	Concentration per 1 mg
B. anthracis LP	1.38×10^6
B. anthracis PP	9.20×10^5
B. subtilis LP	2.50×10^8
B. subtilis PP	2.17×10^9

3.2 Disc Setup

Each disc consisted of 16 channels. Each channel contained a cell line engineered to react with a specific antigen. The cell lines used for the ECBC Phase I testing were

- One *B. subtilis*-specific cell line, designated as Bs
- Two *B. anthracis*-specific cell lines, designated as JC-8 and DH-4

Each disc was used for one sample test and all the discs were loaded with the same pattern of designated cell lines. Each disc contained one positive control (PC) for each cell line and one negative control. Additionally, each disc contained four channels or tests per cell line; therefore, each disc was capable of detecting *B. subtilis* and *B. anthracis*. Table 2 shows the disc setup used in the Phase I testing.

Table 2. Disc Setup

Disc Channel	Cell Line
1	Positive (Bs)
2	Bs
3	JC-8
4	DH-4
5	Positive (JC-8)
6	Bs
7	JC-8
8	DH-4
9	Positive (DH-4)
10	Bs
11	JC-8
12	DH-4
13	Negative Control
14	Bs
15	JC-8
16	DH-4

3.3 White Powder Testing

Six common white powders obtained from the Critical Reagents Program were used for the white powder false positive tests:

- flour
- kaolin
- detergent
- confectionary sugar
- powdered nondairy creamer
- yeast

Prior to testing, 10 mg samples of each powder were weighed and added to individual vials. Triplicate samples of each powder were prepared. Similarly, 10 mg *B. subtilis* PC samples were prepared. One *B. subtilis* PC sample was run after each white powder test to ensure that the instrument could correctly identify the agent. The results showed that the instrument was not affected by the nonhazardous white powder sample.

Before each sample introduction, a BioFlash disc was placed on the opening of the collection apparatus. A nebulizer was used to pretreat the disc with CO_2 independent media for 10 s. To introduce the sample, a macrofoam swab was inserted into a collection cone and then dipped into a vial containing a white powder sample. The disc was then *seeded* by placing the collection cone and swab on the disc for 30 s as the collection apparatus airflow continued. Once seeded, the disc was transferred to the BioFlash Instrument and assayed for the presence of agent (Figure 2).

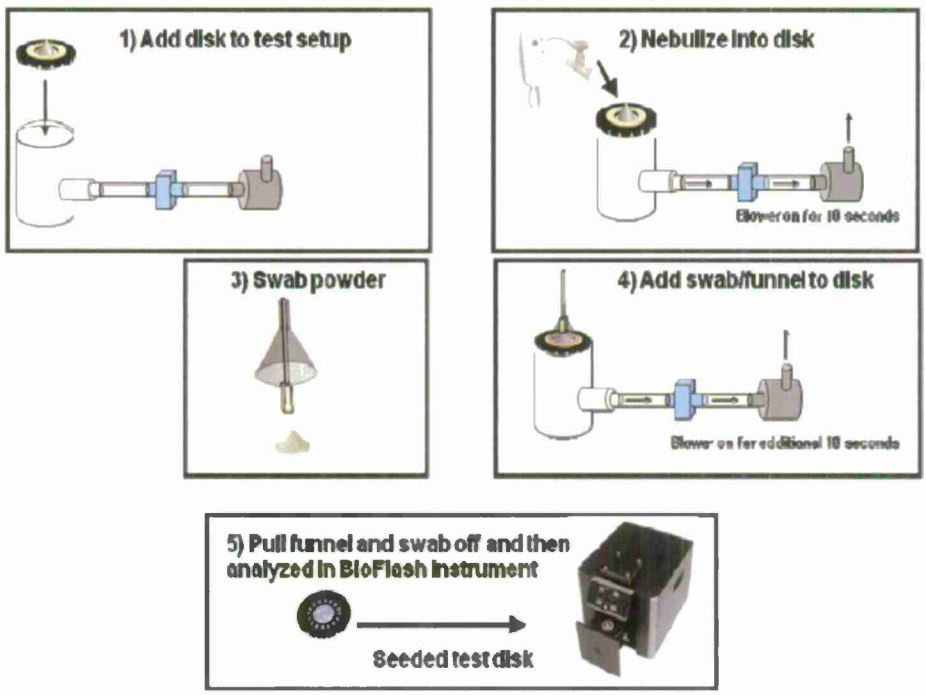

Figure 2. Disc seeding and sample analysis.

3.4 LOD Determination

A series of decreasing weights of spore samples was tested to estimate the BioFlash LOD for live spore powder preparations using the MIT LL collection apparatus. LOD was determined for *B. subtilis* LP, *B. subtilis* PP, *B. anthracis* LP, and *B. anthracis* PP spore preparations. The sample weights tested for each preparation were

- 10 mg
- 1 mg
- 0.1 mg or trace amount (TA)
- Residual amount (RA)

Because the instrumentation used for measuring the powdered spore weights was unable to precisely measure 0.1 mg or less, the 0.1 mg and TA samples were only approximate weights. The TA samples were used for RA testing: a foam swab was used to obtain the RA sample from the TA vial after the TA sample had been tested. The purpose of this sample was to see if any residual powder that was not visible to the naked eye, could be picked up by the swab, introduced into the disk, and subsequently detected by the BioFlash instrument. With the exception of the RA sample, each sample weight was tested in triplicate.

Similar to the white powder testing, the discs were pretreated with CO_2 independent media before sample introduction. The samples were then introduced by inserting the sample vial directly into a collection cone and placing the cone and vial over a disc for 30 s as the collection apparatus continued to pull air. After seeding, the disc was transferred to the BioFlash instrument and assayed for detection of *B. subtilis* or *B. anthracis* spores. After the TA sample was tested, a swab was used to collect any residual powder remaining in the vial. This swab was then placed over the PCA to seed a disc for analysis by the BioFlash instrument.

4. RESULTS AND DISCUSSION

4.1 Results Overview

For each sample, four result indications were possible:

- Agent Found. This occurred if any positive signal on a single agent channel or set of single agent channels was evident.
- No Agent Found. This occurred if no positive signals on any agent channels were evident.
- "PC Failure". This occurred if any PC channel were missed, regardless of whether any of the other channels signaled on the disc.
- "Inconclusive". This occurred if positive signals on both Bs and Ba channels were evident.

The results for each sample tested can be found in Tables 3–7. An analysis of the raw data revealed that all the determinations, except one (sample number 3, a flour sample), appeared to be correct based on the alarms recorded in the "results" computer file. Based on the channels that were triggered, an Agent Found result should have been indicated instead of an Inconclusive result for sample number 3.

4.2 Effect of Machine Errors on Disc Results

Several anomalous indications made during testing were due to the BioFlash detector, which had three mechanical failures. Two of the failures resulted in error messages: one "Communication Error" message and one "Spin Pro Error" message. The Spin Pro Error message likely caused the single "No Determinate" indication for the *B. anthracis* residual sample. The RA sample was repeated and the result was a successful Agent Found indication by the detector. For the Communication Error message, function was restored after the BioFlash instrument was powered down and then powered back up. The third failure resulted in PC Failure indications for sample numbers 65–68 because the BioFlash photo multiplier tube malfunctioned and failed to open. This was noticed when the expected background signals did not register on the data log. Data collected from these test discs showed only "0" values. The issue could not be resolved; therefore, the instrument was replaced with a new BioFlash system and these sample results were recorded as "Error" calls.

4.3 Effect of Disc Assembly Errors on Disc Results

The PC samples for the Phase 1 testing, prepared manually by IBI personnel, were not subjected to QA/QC oversight because there were no measures in place to ensure the discs were properly seeded; therefore, human error in the tests is a possibility. In several cases, it appears that an inadequate level of PC material was applied to the disc prior to testing. This resulted in a PC Failure indication for discs 23, 32, 33, and 34. PC Failure due to malfunctioning of the B cells for these discs is highly unlikely. Analysis of the signals for the remaining agent channels revealed that the correct result could have been made for each of these samples.

4.4 Nonhazardous White Powder Testing Results

For the white powder false positive testing, 17 of the 36 samples resulted in unexpected detection indication results. Of these, six were Inconclusive, four were Agent Found, and seven were PC Failure. The results for each sample are found in Table 3, which shows that flour, kaolin, and alconox triggered unexpected results.

Table 3. Suspect White Powder Testing

Sample Number	Suspect Powder	Expected Result	Actual Result
1	Flour	No Agent Found	Inconclusive*
2	*B. subtilis*	Agent Found	Agent Found
3	Flour	No Agent Found	Inconclusive*
4	*B. subtilis*	Agent Found	Agent Found
5	Flour	No Agent Found	Agent Found*
6	*B. subtilis*	Agent Found	Agent Found
7	Kaolin	No Agent Found	Agent Found*
8	*B. subtilis*	Agent Found	Inconclusive*
9	Kaolin	No Agent Found	Agent Found*
10	*B. subtilis*	Agent Found	Agent Found
11	Kaolin	No Agent Found	Agent Found*
12	*B. subtilis*	Agent Found	Inconclusive*
13	Alconox	No Agent Found	PC Failure*
14	*B. subtilis*	Agent Found	Agent Found
15	Alconox	No Agent Found	PC Failure*
16	*B. subtilis*	Agent Found	Agent Found
17	Alconox	No Agent Found	PC Failure*
18	*B. subtilis*	Agent Found	Agent Found
19	Confectionary Sugar	No Agent Found	No Agent Found
20	*B. subtilis*	Agent Found	Inconclusive*
21	Confectionary Sugar	No Agent Found	No Agent Found
22	*B. subtilis*	Agent Found	Inconclusive*
23	Confectionary Sugar	No Agent Found	PC Failure*
24	*B. subtilis*	Agent Found	Agent Found
25	Nondairy creamer	No Agent Found	No Agent Found
26	*B. subtilis*	Agent Found	Agent Found
27	Nondairy creamer	No Agent Found	No Agent Found
28	*B. subtilis*	Agent Found	Agent Found
29	Nondairy creamer	No Agent Found	No Agent Found
30	*B. subtilis*	Agent Found	Agent Found
31	Yeast	No Agent Found	No Agent Found
32	*B. subtilis*	Agent Found	PC Failure*
33	Yeast	No Agent Found	PC Failure*
34	*B. subtilis*	Agent Found	PC Failure*
35	Yeast	No Agent Found	No Agent Found
36	*B. subtilis*	Agent Found	Agent Found

*Represents an unexpected result

4.4.1 Results for Flour

Testing of each flour sample resulted in either an Inconclusive or Agent Found indication. For the flour samples resulting in Inconclusive determinations, there were positive signals on all PC channels and on one or more of the Bs channels. The signal on the Bs channels seemed real, and it is unlikely that it could have been removed by alteration of the algorithm without significantly affecting the sensitivity of agent detection. There was also one positive signal on a Ba (DH-4) channel resulting in an Inconclusive result. The signal on the Ba channel was significantly lower than the signals on the Bs channels, and it was removed by increasing the stringency of the algorithm.

For the sample disc, which resulted in an Agent Found indication, there were positive signals on all the PC channels and three of the four Bs channels. Again, the positive signals on the Bs channels appeared to be real, and it is unlikely that they could have been removed by altering the algorithm without impacting its sensitivity. One possible explanation for the positive signals could be that the flour samples contained *B. subtilis* spores because Bs is a common contaminant found in flour (Bailey et al., 1993; Pepe et al., 2003; Sorokulova et al., 2003; and Te Giffel et al., 1996).

4.4.2 Results for Kaolin

For each kaolin sample tested, the actual result was Agent Found. This was due to positive signals on the Ba JC-8 or DH-4 channels. In all the cases, the curve of the light output generated on these channels was the incorrect shape for consideration as a true positive, and it could be removed by increasing the stringency of the algorithm.

4.4.3 Results for Alconox

Analysis of the the alconox samples showed multiple problems with the B cell channels. They had high, rapid output of light, which obscured the real PC signals (hence, their PC Failure indication). The B cell channels also produced high light output signals for agent channels that would have resulted in Agent Found indications if all of the PC channels had been detected.

It is unlikely that the confounding effects of this agent on the B cells could have been overcome via modification of the algorithm. Many detergents have deleterious effects on the B cells because they change membrane permeability properties.

4.4.4 *B. subtilis* PCs

As specified in the test plan for this study, positive *B. subtilis* samples were run after each nonhazardous powder introduction. This ensured that the nonhazardous white powder samples did not have deleterious effects on the equipment, which would have prevented the system from effectively detecting positive samples after these substances were introduced into the system. During the testing of the nonhazardous powders, the introduction of several *B. subtilis* PC samples triggered Inconclusive determinations. This occurred with sample numbers 8, 12, 20, and 22 (see Table 3). For each of these samples, the results showed that all PC channels

produced positive signals. All Bs channels also produced positive signals. In each case, there was one nonspecific positive response on a Ba JC8 cell-line channel. Analysis of this data revealed that the signal on this channel was very low in each case, and it could be removed by increasing the stringency of the algorithm.

4.5 LOD Results

For the LOD determination, 12 of the 54 samples tested resulted in unexpected indications. Of these, four were Inconclusive, one was Agent Found, two were No Agent Found, four were PC Failure, and one was a No Determinate result. The results for each LOD test can be found in Tables 4–7.

Analysis of the LOD testing results indicated that the system could be used to detect known agent samples at all sample test weight levels. For three of the four RA samples tested, agent was detected by the system. Of all LOD samples tested, only one known agent sample resulted in a No Agent Found indication. Five known agent samples resulted in Inconclusive indications, but further analysis of the data revealed that in all but one case, the correct channels were triggered for the specific agent tested. However, an incorrect channel was also triggered, which resulted in the erroneous result.

A comparison of the LPs and PPs for each organism reveals slight differences in the detection ability of the system for each one. However, these differences were not consistent among the two organisms. For example, for *B. subtilis*, it appeared that the PP was slightly more problematic to detect than the LP. Except for *B. anthracis*, the LP appeared to be more problematic. It is worth noting that this is only an observation and that the small sample sizes tested in these studies do not allow definitive comparisons of the spore preparations.

Table 4. LOD for *B. subtilis* LP

Sample ID	Agent	Concentration (mg)	Expected Result	Actual Result
37	*B. subtilis* LP	10	Agent Found	Agent Found
38	*B. subtilis* LP	10	Agent Found	Agent Found
39	*B. subtilis* LP	10	Agent Found	Agent Found
40	Negative	--	No Agent Found	No Agent Found
41	*B. subtilis* LP	1	Agent Found	Agent Found
42	*B. subtilis* LP	1	Agent Found	Agent Found
43	*B. subtilis* LP	1	Agent Found	Agent Found
44	Negative	--	No Agent Found	No Agent Found
45	*B. subtilis* LP	TA	Agent Found	Agent Found
46	*B. subtilis* LP	TA	Agent Found	Agent Found
47	*B. subtilis* LP	TA	Agent Found	Agent Found
48	*B. subtilis* LP	RA	Agent Found	Agent Found

Table 5. LOD for *B. subtilis* PP

Sample ID	Agent	Concentration (mg)	Expected Result	Actual Result
49	*B. subtilis* PP	10	Agent Found	Inconclusive*
50	*B. subtilis* PP	10	Agent Found	Agent Found
51	*B. subtilis* PP	10	Agent Found	Agent Found
52	Negative	--	No Agent Found	No Agent Found
53	*B. subtilis* PP	1	Agent Found	Agent Found
54	*B. subtilis* PP	1	Agent Found	Agent Found
55	*B. subtilis* PP	1	Agent Found	Inconclusive*
56	Negative	--	No Agent Found	No Agent Found
57	*B. subtilis* PP	TA	Agent Found	Agent Found
58	*B. subtilis* PP	TA	Agent Found	Agent Found
59	*B. subtilis* PP	TA	Agent Found	Agent Found
60	*B. subtilis* PP	RA	Agent Found	No Agent Found*

*Unexpected Results

Table 6. LOD for *B. anthracis* LP

Sample ID	Agent	Concentration (mg)	Expected Result	Actual Result
61	*B. anthracis* LP	10	Agent Found	Agent Found
62	*B. anthracis* LP	10	Agent Found	Agent Found
63	*B. anthracis* LP	10	Agent Found	Agent Found
64	Negative	--	No Agent Found	Agent Found*
65	*B. anthracis* LP	1	Agent Found	Error*+
66	*B. anthracis* LP	1	Agent Found	Error*+
67	*B. anthracis* LP	1	Agent Found	Error*+
68	Negative	--	No Agent Found	Error*+
69	*B. anthracis* LP	1	Agent Found	Inconclusive*
70	*B. anthracis* LP	1	Agent Found	Inconclusive*
71	*B. anthracis* LP	1	Agent Found	Agent Found
72	Negative	--	No Agent Found	No Agent Found
73	*B. anthracis* LP	TA	Agent Found	Agent Found
74	*B. anthracis* LP	TA	Agent Found	No Agent Found*
75	*B. anthracis* LP	TA	Agent Found	Agent Found
76	Negative	--	No Agent Found	No Agent Found
77	*B. anthracis* LP	RA	Agent Found	Agent Found
78	*B. anthracis* LP	RA	Agent Found	Agent Found

*Unexpected results
+PhotoMultiplier tube malfunction

Table 7. LOD for *B. anthracis* PP

Sample ID	Agent	Concentration (mg)	Expected Result	Actual Result
79	*B. anthracis* PP	10	Agent Found	Agent Found
80	*B. anthracis* PP	10	Agent Found	Agent Found
81	*B. anthracis* PP	10	Agent Found	Agent Found
82	Negative	--	No Agent Found	No Agent Found
83	*B. anthracis* PP	1	Agent Found	Agent Found
84	*B. anthracis* PP	1	Agent Found	Agent Found
85	*B. anthracis* PP	1	Agent Found	Agent Found
86	Negative	--	No Agent Found	No Agent Found
87	*B. anthracis* PP	TA	Agent Found	Agent Found
88	*B. anthracis* PP	TA	Agent Found	Agent Found
89	*B. anthracis* PP	TA	Agent Found	Agent Found
90	*B. anthracis* PP	RA	Agent Found	No Determinate* Indication
91	*B. anthracis* PP	RA	Agent Found	Agent Found

*Unexpected results

4.5.1 *B. subtilis* LOD Tests

Similar to the nonhazardous white powder testing, there were two samples, numbers 49 and 55, of the *B. subtilis* PP preparation, which resulted in Inconclusive indications during the LOD testing (Table 5). These results occurred in the 10 and 1 mg samples of *B. subtilis*. In these discs, all PC and Bs channels produced positive signals. There was one nonspecific positive response on a Ba JC8 cell-line channel. Again, it was determined that because the signal on this channel was very low in both cases, the erroneous indications could be removed by increasing the stringency of the algorithm.

4.5.2 Negative Controls

One negative control (sample 64), assayed during the LOD, testing resulted in an Agent Found indication (Table 5). Analysis of the raw data for this sample showed that all PC channels produced positive signals. Although one Ba JC8 channel produced a positive response, no clear light output curve was visible in the light output data. By increasing the stringency of the algorithm, this signal could be excluded.

4.5.3 *B. anthracis* LOD Tests

For the Ba LOD testing, two samples (69 and 70) resulted in Inconclusive indications, (Table 6). For these sample discs, all PC and Ba channels produced positive signals. In each case, one Bs channel produced a low positive signal. Again, this low signal could be excluded by increasing the stringency of the algorithm.

Sample 74, a TA sample of the Ba LP, resulted in a No Agent Found indication. When testing this disc, all PC channels produced positive signals. No other positive signals were produced on any agent channels. It is likely that the amount of agent collected on the disc, or the concentration tested, was below the LOD for this agent.

5. CONCLUSIONS

Key observations from the Phase I testing are summarized as follows:

- The MIT LL PCA was effectively used to collect the powdered samples into the test discs. This assessment was based on the ability of the system to successfully detect known agent samples.

- Low levels of known agent were consistently detected using the system; this includes residual or nonvisible samples.

- Certain nonhazardous white powders proved problematic for the use of this detection system and triggered false positive responses or resulted in Inconclusive indications.

Several general improvements in algorithm function have been made by IBI in the succeeding months. Many algorithm parameters used in the BioFlash instrument are tunable, allowing modification of the algorithm function for specific applications. The algorithm installed on the BioFlash units at the time of the ECBC testing was primarily designed for maximum sensitivity. Post-testing analyses of the data showed that the signals generated during testing were of a higher magnitude than expected. Thus, nonspecific channels were detected as positive, which produced Inconclusive indications. By resetting the algorithm parameters to make the algorithm determinations more stringent, IBI was able to dramatically reduce the number of Inconclusive indications and produce more accurate results. Because the entire set of data was not run through the modified algorithm, it was not possible to predict the impact that such modifications had on the study determinations, which were made as expected. It would be useful to run the entire set of data files from the ECBC tests through the modified algorithm to determine the utility of this improved version for future testing.

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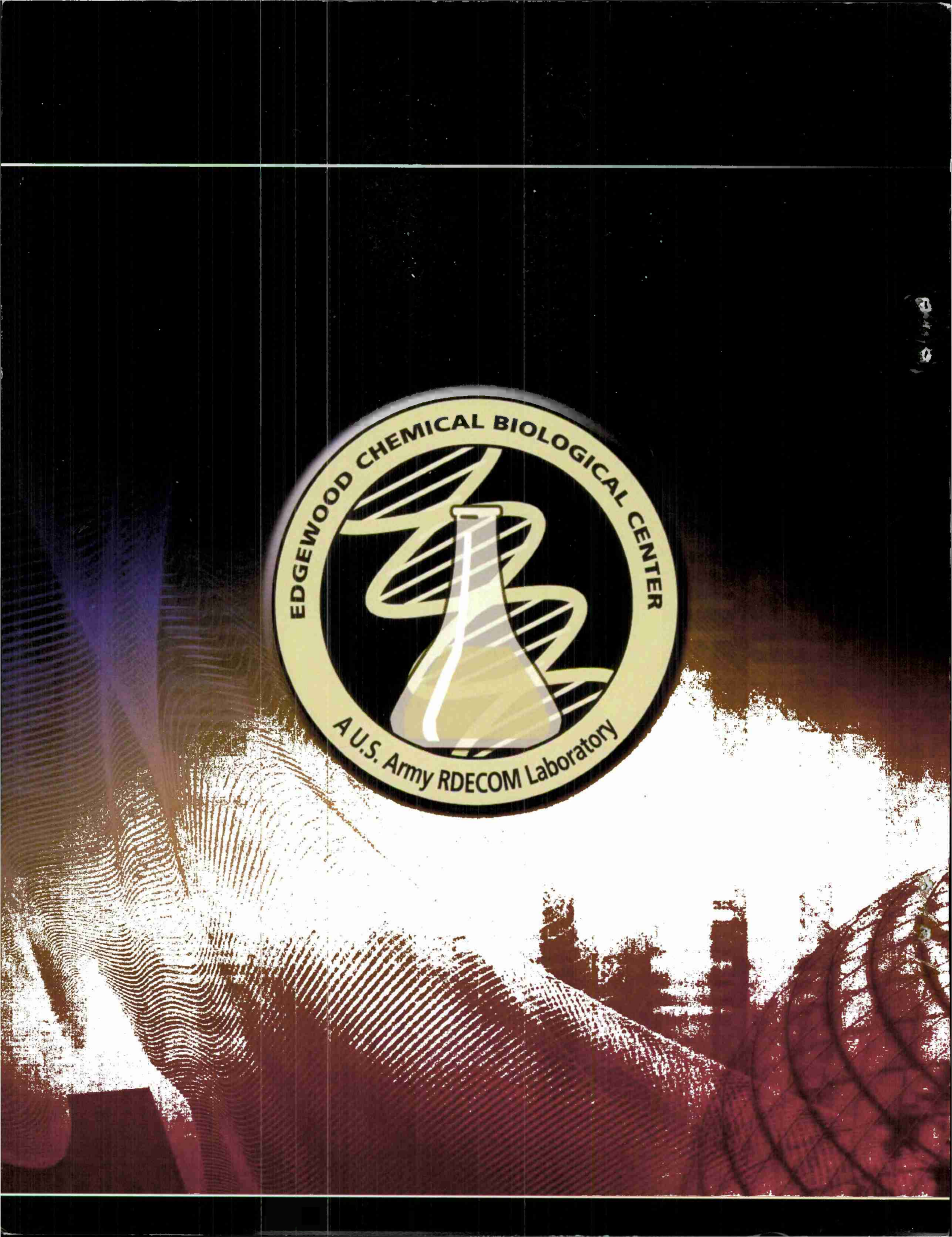
GLOSSARY

CANARY	cellular analysis and notification of antigen risks and yields
DHS S&T	U.S. Department of Homeland Security Science and Technology Directorate
ECBC	U.S. Army Edgewood Chemical Biological Center
FFT	fast Fourier transform
IBI	Innovative Biosensors, Inc.
LOD	limit of detection
LP	lesser preparation
MIT LL	Massachusetts Institute of Technology Lincoln Laboratory
PBS	phosphate buffered saline
PC	positive control
PCA	powder collection apparatus
PP	professional preparation
QA/QC	Quality Assurance/Quality Control
RA	residual amount
TA	trace amount
TSA	tryptic soy agar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